CHAPTER 2

3.5 Billion Years of Mechanosensory Transduction: Structure and Function of Mechanosensitive Channels in Prokaryotes

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I. OVERVIEW

Over the last decade, studies of prokaryotic mechanosensitive (MS) ion channels have been at the forefront of the MS channel research field. Two major events that greatly advanced the research on prokaryotic MS channels after their initial discovery in *Escherichia coli* some 20 years ago, consisted of cloning MscL and MscS, the bacterial MS channels of *L*arge and *S*mall conductance, and solving their three-dimensional (3D) crystal structure. These key events were followed by cloning and molecular characterization of MS channels in archaea, which has since made possible further exploration of the phylogenetic relationship and common structural and functional properties in prokaryotic MS channels. Moreover, these promising developments have significantly contributed to our understanding of basic physical principles and evolutionary origins of the mechanosensory transduction in living organisms.

II. INTRODUCTION

As the primary molecular transducers of mechanical force in living cells MS ion channels have been implicated in a myriad of mechanosensory physiological processes. Touch, hearing, and blood pressure control are just a few examples of these processes (Sachs and Morris, 1998; Hamill and Martinac, 2001; Martinac, 2004; Sukharev and Corey, 2004). In the life of a prokaryotic cell, MS channels are indispensable for survival when the external environment becomes hypoosmotic relative to the cell interior and increase in cellular turgor threatens to kill the microbe. Studies of MS channels in bacteria and archaea (Fig. 1) became possible with the advent of the patch-clamp technique (Hamill *et al.*, 1981), which has removed the constraint of being able to study electrophysiologically only cells that are large enough to be impaled with glass microelectrodes.

Since their discovery in *E. coli* (Martinac *et al.*, 1987), MS channels have extensively been studied in both Gram-negative and Gram-positive bacteria (Martinac *et al.*, 1992; Zoratti and Ghazi, 1993; Blount *et al.*, 1999). Their existence has also been documented in cell membranes of archaea (Kloda and Martinac, 2001a), which form a separate prokaryotic kingdom (Fig. 1). Studies of archaeal MS channels began with electrophysiological characterization of MS channels in the halophilic archaeon *Haloferax volcanii* (formerly *Halobacterium volcanii*) (Le Dain *et al.*, 1998) followed by molecular cloning and characterization of MS channels in methanogenic *Methanococcus jannaschii* (Kloda and Martinac, 2001b,c) and thermophilic

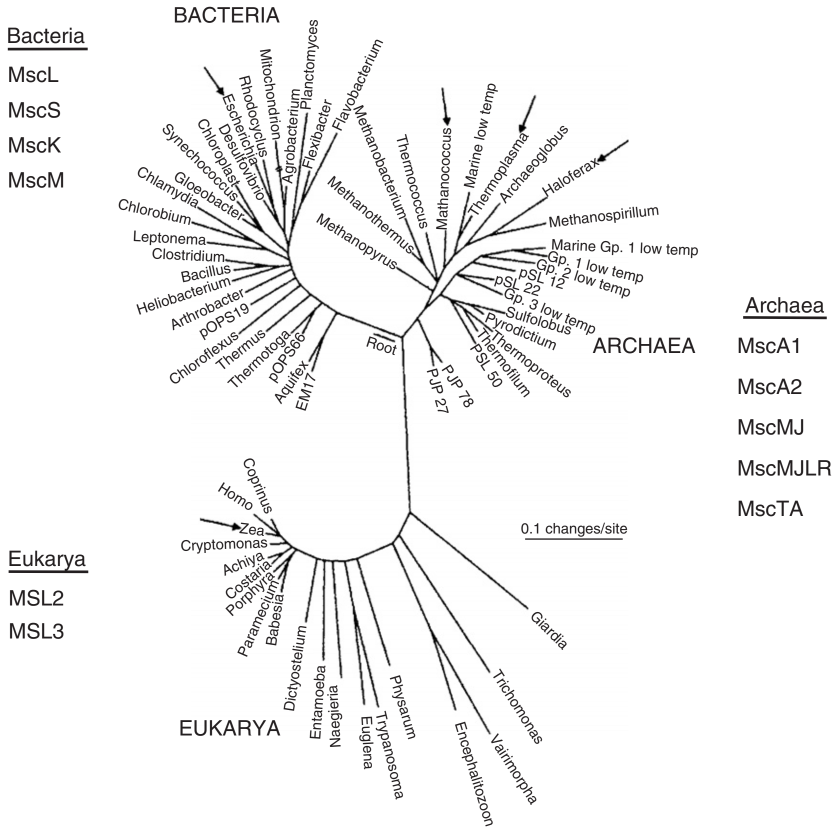


FIGURE 1 Universal phylogenetic tree showing the life on Earth organized in three kingdoms of living organisms based on small subunit tRNA sequences (modified from Pace, 1997; with permission). Prokaryotic MS channels that have been identified and characterized are listed next to the group of organisms in which they are found (indicated by arrows).

Thermoplasma acidophilum (Kloda and Martinac, 2001d). The existence and close structural and functional similarities of MS channels in cells belonging to both prokaryotic kingdoms suggest that this class of ion channels appeared very early during the evolution of life on Earth (Martinac, 1993; Kung and Saimi, 1995).

For interested readers, a number of reviews will provide information on prokaryotic MS channels not covered here (Perozo and Rees, 2003; Strop *et al.*, 2003; Anishkin and Kung, 2005; Blount *et al.*, 2005; Sukharev *et al.*, 2005; Perozo, 2006).

III. DISCOVERY, MECHANISM, AND STRUCTURE OF MS CHANNELS IN PROKARYOTES

A. Historical Perspective

Microbes, including bacteria, have often been used to advance our knowledge of basic principles underlying diverse aspects of cellular biology. A Nobel Prize-winning structural characterization and mechanistic studies of bacterial ion channels (Doyle *et al.*, 1998; Dutzler *et al.*, 2002; Jiang *et al.*, 2002) is a good example, which made scientific community realize that bacteria have ion channels that could be used to advance knowledge of eukaryotic channels far beyond what is achievable using traditional functional approaches alone. A comprehensive summary of the current knowledge on bacterial ion channels can be found in the published book on "Bacterial ion channels and their eukaryotic homologues" (Kubalski and Martinac, 2005).

Studies of MS channels in bacteria were facilitated by the advent of the patch-clamp technique (Hamill *et al.*, 1981), which allowed electrophysiologists to examine cells of almost any size. Nevertheless, patch-clamping bacterial cells presented as before the ultimate technical challenge. Overcoming this challenge promised to provide a fertile ground for functional and structural characterization of ion channels because of a wealth of available information on biochemistry, genetics, and molecular biology of these microorganisms. The initial survey of a bacterial cell membrane by the patch clamp became possible with development of a "giant spheroplast" preparation of *E. coli* (Ruthe and Adler, 1985; Martinac *et al.*, 1987). This technical advance has led to a discovery of prokaryotic MS channels and opened a window of opportunities for structure and function studies of this class of membrane proteins (Fig. 2). Given that bacteria offer distinct experimental advantages including short doubling time and large yield, the advantage of using bacteria for structural studies in MS channels is obvious. Bacteria can be grown in large quantities delivering milligram amounts of channel proteins required for structural studies by X-ray crystallography. In fact, 3D structures of two bacterial MS channels, MscL from *Mycobacterium tuberculosis* (Chang *et al.*, 1998) and MscS from *E. coli* (Bass *et al.*, 2002), were solved just a few years after cloning of these MS channels in *E. coli* (Sukharev *et al.*, 1994a; Levina *et al.*, 1999).

B. Conductance, Selectivity, and Activation by Membrane Tension of Bacterial MS Channels

Among prokaryotic MS channels studied to date, the best characterized are the MS channels of *E. coli*, which harbors three types of MS channels in its cytoplasmic membrane, based on their conductance and sensitivity to

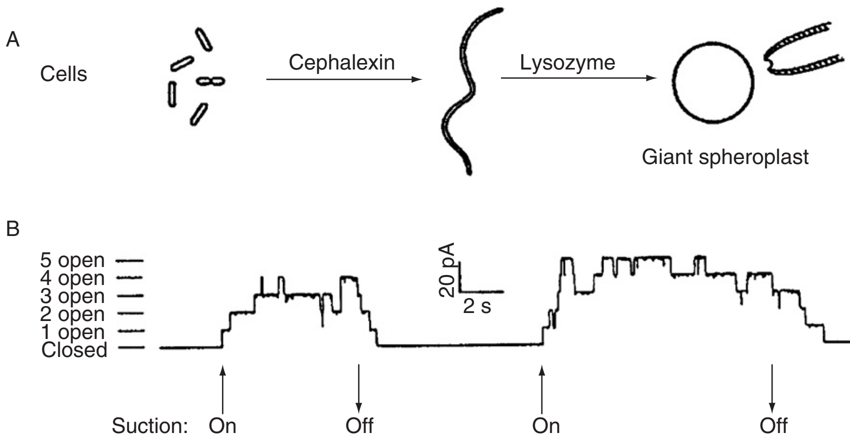


FIGURE 2 MS channels of small conductance were discovered first. They have extensively been studied in giant spheroplasts of *E. coli.* (A) A bacterial cell in the presence of the antibiotic cephalexin fails to septate and grows into a long filament. When treated with EDTA and lysozyme the filament transforms into a giant spheroplast of 5–10 μm in diameter, which can be examined for channel activity by the patch clamp. (B) Activities of up to five MS channels of small conductance (~1 nS) were recorded from a patch of a giant spheroplast. Channels opened on suction applied (on) to the patch-clamp pipette and closed when suction was released (off). Adapted from Martinac *et al.* (1987).

applied pressure: (i) MscM (M for mini), (ii) MscS (S for small) and MscK (K for kalium, i.e., potassium), and (iii) MscL (L for large) (Berrier *et al.*, 1996).

MscS and MscK present the first type of bacterial MS channel characterized by the patch clamp (Martinac *et al.*, 1987). Initially, they were considered to represent a single type of a bacterial MS channel because in patch-clamp experiments they exhibited similar activation by pressure and had a conductance of ~1 nS (Fig. 2) (Martinac *et al.*, 1987; Sukharev *et al.*, 1993). However, independent studies have shown that their activities could clearly be distinguished (Li *et al.*, 2002). In contrast to MscK (and MscL), MscS exhibits a tension-dependent adaptation/inactivation (Koprowski and Kubalski, 1998). Furthermore, a distinguishing property of MscK is its sensitivity to the extracellular ionic environment (Li *et al.*, 2002). The overall similarities, however, between MscS and MscK point to their structural similarity, which became apparent after these two channels have been cloned (Levina *et al.*, 1999). MscK contains an MscS-like domain at its C-terminus. The conductance of MscS is about 1 nS, which is roughly one third of that of MscL (~3 nS) (Sukharev *et al.*, 1993), whereas the unitary conductance of MscM is about one third to one half of that of MscS, that is, ~0.3 nS

(Berrier *et al.*, 1996). The activity of MscM is less frequently encountered in membrane patches of giant spheroplasts compared to those of MscS or MscL (Cui *et al.*, 1995). The relation between conductance and pressure sensitivity of the MscM, MscS/MscK, and MscL channels is such that the higher the conductance, the higher negative pressure is required for the channel activation.

MS channels of *E. coli* differ not only in their sensitivity to membrane tension but also in terms of their ionic preference. MscL is nonselective for both anions and cations (Sukharev *et al.*, 1993; Cruickshank *et al.*, 1997), whereas MscS exhibits a slight preference for anions over cations with a permeability ratio $P_{Cl^-}/P_{K^+} \sim 1.5 - 3.0$ (Martinac *et al.*, 1987; Sukharev *et al.*, 1993; Sukharev, 2002). In addition, MscS exhibits rectifying properties such that in a symmetric solution its conductance is approximately one-third bigger at positive compared to that at negative pipette voltages (Martinac *et al.*, 1987; Sukharev *et al.*, 1993). MscK was also reported to show some anionic preference (Li *et al.*, 2002), although mutational analysis has suggested that it could be cation specific (McLaggan *et al.*, 2002) (Table I).

In situ in giant spheroplasts, the ratio of the pressure threshold of activating MscL by membrane tension to that of MscS is ~1.5 (Blount *et al.*, 1996; Iscla *et al.*, 2004). Both channels can also be activated by osmotic force (Martinac *et al.*, 1992; Cui *et al.*, 1995). In difference to MscL, MscS is also voltage dependent. The activity of the channel increases with membrane depolarization (Martinac *et al.*, 1987; Sukharev, 2002). However, voltage alone has not been shown to activate MscS, but is rather acting in synergy with the channel mechanosensitivity by exerting a modulatory effect on the MscS channel activated by membrane tension. Summary of properties of the prokaryotic MS channels is given in Table I.

C. Cloning of MscL and MscS of E. coli

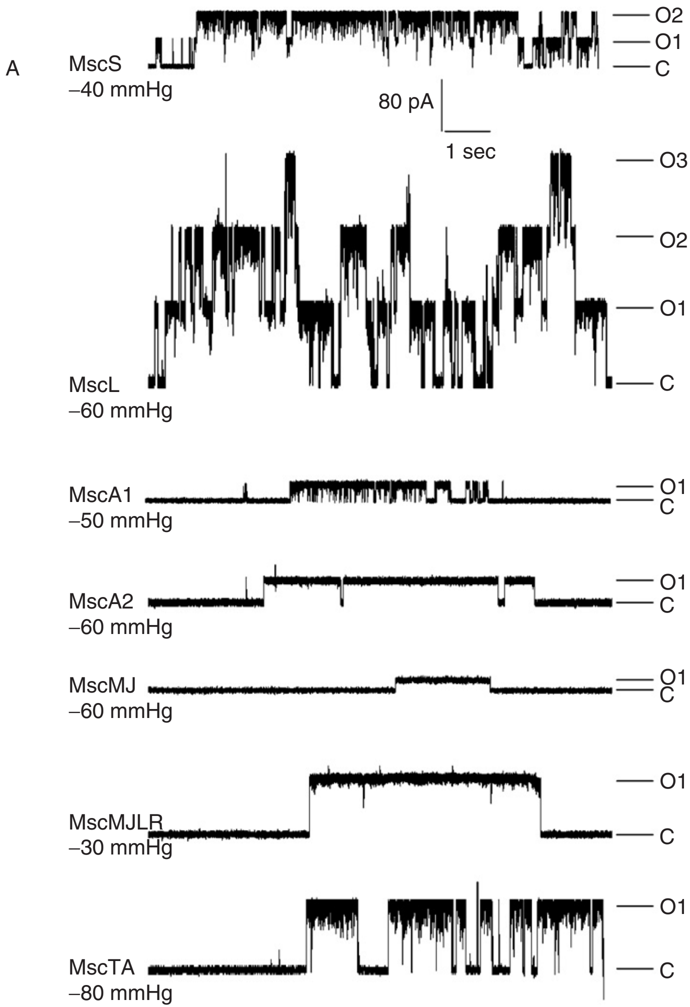
The cloning strategy of bacterial MS channels differed for MscL and MscS/MscK. For the *mscL* gene, which was cloned by Kung and coworkers (Sukharev *et al.*, 1994a), the strategy was quite unusual. Activation of MscL by membrane tension in the lipid bilayer (Fig. 3) was essential for the strategy, which allowed for detergent solubilization, fractionation of *E. coli* membrane constituents by column chromatography and functional examination of the individual fractions for MS channel activity by the patch clamp (Sukharev *et al.*, 1993). This approach made possible identification of the MscL protein, which further enabled the cloning of the corresponding *mscL* gene (Sukharev *et al.*, 1994a,b). The expression of the *mscL* gene in a

TABLE I

Summary of Basic Properties of Prokaryotic MS Channels That Have Been Characterized Structurally, Functionally, or Both[a]

MS channel	Source	Conductance (nS)	d_{pore} (Å)	Selectivity	Amphipaths	ΔG (kT)	References
MscL	Bacteria	3.3–3.8	~30	Nonselective	CPZ, TNP, LPC	14–19	Häse *et al.*, 1995; Cruickshank *et al.*, 1997; Sukharev *et al.*, 1999; Kloda and Martinac, 2001d; Perozo *et al.*, 2002a
MscS	Bacteria	0.97 (+ve) 0.65 (–ve)	18	$P_{Cl^-}/P_{K^+} \sim 1.5 - 3.0$	CPZ, TNP, LPC, local anesthetics	7	Martinac *et al.*, 1987; Martinac *et al.*, 1990; Sukharev *et al.*, 1993; Kloda and Martinac, 2002; Sukharev, 2002
MscM	Bacteria	~1	ND	$P_{Cl^-}/P_{K^+} > 1$	ND	ND	Li *et al.*, 2002; Blount *et al.*, 2005
MscA1	Archaea	0.38 (+ve) 0.68 (−ve)	11	ND	ND	15	Le Dain *et al.*, 1998
MscA2	Archaea	0.85 (+ve) 0.49 (−ve)	17	ND	ND	29	Le Dain *et al.*, 1998
MscMJ	Archaea	0.27	9	$P_{K^+}/P_{Cl^-} \sim 6$	CPZ, TNP	5	Kloda, 2001; Kloda and Martinac, 2001b
MscMJLR	Archaea	2.2 (+ve) 1.7 (−ve)	27	$P_{K^+}/P_{Cl^-} \sim 5$	Not affected by CPZ or TNP	18	Kloda, 2001; Kloda and Martinac, 2001c
MscTA	Archaea	2.8	ND	Nonselective	TNP	35	Kloda and Martinac, 2001d; Kloda and Martinac, 2002
MSL2	Plants	ND	ND	ND	ND	ND	Haswell and Meyerowitz, 2006
MSL3	Plants	ND	ND	ND	ND	ND	Haswell and Meyerowitz, 2006

[a]ND indicates that a particular property has not been determined.
Abbreviations: CPZ, chlorpromazine; TNP, trinitrophenol; LPC, lysophosphatidylchlone.


A
MscS
–40 mmHg
O2
O1
C
80 pA
1 sec
O3
O2
O1
MscL
–60 mmHg
C
MscA1
–50 mmHg
O1
C
MscA2
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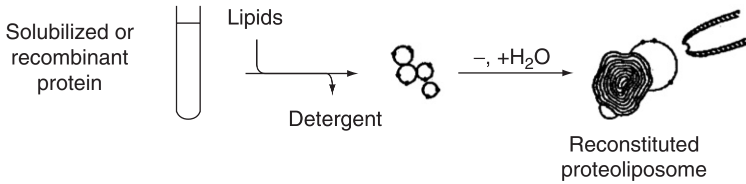
B
Solubilized or recombinant protein
Lipids
Detergent
–, +H2O
Reconstituted proteoliposome

heterologous as well as *in vitro* transcription/translation system demonstrated that the *mscL* gene alone is necessary and sufficient for the MscL activity. Since its discovery, genes homologous to *mscL* were found in various Gram-negative and Gram-positive bacteria, archaea and a single fungal genome (Kloda and Martinac, 2002; Martinac and Kloda, 2003; Pivetti *et al.*, 2003).

MscS and MscK were cloned by Booth and coworkers (Booth and Louis, 1999; Levina *et al.*, 1999), who identified two genes on *E. coli* chromosome, yggB and kefA. Deletion of the two genes led to the abolishment of the activity of the MS channels of small conductance, which was originally described as a single type of MS channel in bacterial spheroplasts (Martinac *et al.*, 1987). The MS channels affected by the kefA and yggB null mutations correspond to MscK and MscS, respectively. The MscS channel activity is characterized by a large number of channels gating simultaneously encountered in almost 100% of spheroplast patches. MscS inactivates rapidly on sustained application of pressure (Koprowski and Kubalski, 1998). The activity of the KefA channels is less frequently encountered (70% of the patches). It is characterized by fewer channels, which do not inactivate on prolonged application of pressure to the patch pipette. YggB is a small membrane protein of 286 amino acids. In contrast, KefA is about five times larger, multidomain membrane protein of 1120-amino acid residues. The primary amino acid sequence of YggB resembles highly the sequence of the last two domains of the KefA protein.

D. Molecular Identification of MS Channels in Archaea

Archaea, formerly referred to as archaebacteria, are prokaryotes like bacteria. They exist in extreme environments found on Earth (Barinaga, 1994) and constitute a separate domain on the phylogenetic tree (Fig. 1) (Woese, 1994; Pace, 1997). The existence of MS channels in archaea was first documented in the halophilic archaeon *Haloferax volcanii* (Le Dain *et al.*, 1998) followed by molecular identification and functional characterization

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FIGURE 3 Multiplicity of MS channels in prokaryotes. (A) Shown are current traces *E. coli* MscL and MscS, followed by traces of MscA1 and MscA2 of *Haloferax volcanii* and MscMJ and MscMJLR of *Methanococcus jannaschii* recorded from channels reconstituted into liposomes. The last current trace represents activity of MscTA, the channel of *T. acidophilum*. All traces were recorded at +40 mV at negative pipette pressures indicated on the left of each trace. C denotes the closed state and O_n denotes open state of n channels. (B) A scheme of a dehydration/rehydration method used for liposome reconstitution of MS channels. Note: 1 mm Hg = 133 Pa. Reproduced from Martinac and Kloda (2003).

of MS channels in methanogenic archaeon *Methanococcus jannaschii* (Kloda and Martinac, 2001b,c) and in two thermophilic archaea *T. volcanium* and *T. acidophilum* (Fig. 1) (Kloda and Martinac, 2001d).

MscA1 and MscA2 are two types of MS channels found in the cell membrane of *Haloferax volcanii* (Le Dain *et al.*, 1998) (Fig. 1). Both channels have large conductance, rectified with voltage, and are blocked by submillimolar concentrations of the lanthanide Gd^{3+}, a common blocker of MS channels (Sachs and Morris, 1998; Hamill and Martinac, 2001) (Table I). Similar to the bacterial MS channels, they are activated solely by tension in the lipid bilayer. Consequently, in patch-clamp experiments they fully preserve their mechanosensitivity after detergent solubilization and reconstitution into artificial liposomes (Fig. 3).

Two types of MS channels, MscMJ and MscMJLR, have been identified in the genome of *Methanococcus jannaschii* (Kloda and Martinac, 2001b,c). The primary amino acid sequence of MscMJ shares high homology with MscS of *E. coli* (Levina *et al.*, 1999). The channel has conductance of 270 pS and prefers cations to anions with a selectivity characterized by $P_{K^+}/P_{Cl^-} \sim 6$. Its activation by membrane tension is comparable to the MscS activation (Table I). MscMJLR (i.e., MS channel of *Methanococcus jannaschii* of large conductance and rectifying) is a second MS channel of *Methanococcus jannaschii*, which was identified and functionally characterized shortly after MscMJ was described (Kloda and Martinac, 2001b). Like MscMJ, MscMJLR shares sequence homology with a large group of MscS-like proteins identified in prokaryotic microbes as well as in eukaryotic organisms including the experimental plant *Arabidopsis thaliana* and fission yeast *Schizosaccharomyces pombe* (Kloda and Martinac, 2002; Pivetti *et al.*, 2003). MscMJLR is cation selective with the permeability ratio $P_{K^+}/P_{Cl^-} \sim 5$ comparable to the selectivity of MscMJ. However, MscMJLR differs from MscMJ in both conductive and MS properties. Comparable to MscL of *E. coli*, MscMJLR has a very large conductance of ~2.0 nS that is approximately seven times larger than the 270-pS conductance of MscMJ. It also requires much higher membrane tension for activation (Kloda and Martinac, 2001c) (Table I). MscMJLR is also blocked by submillimolar concentrations of Gd^{3+} comparable to other prokaryotic MS channels (Kloda, 2001; Kloda and Martinac, 2002). Interestingly, the amino acid sequence of the third membrane-spanning domain TM3 of MscMJ and MscMJLR resembles the sequence of the highly conserved TM1 helix of MscL. This is important because TM1 is the helix essential for the opening of the MscL pore by membrane tension (Yoshimura *et al.*, 1999; Ajouz *et al.*, 2000; Betanzos *et al.*, 2002; Perozo *et al.*, 2002a). The presence of multiple MS channels in prokaryotic cells indicates the importance of MS channels

for the survival of these microbes being frequently exposed to environmental osmotic challenges.

The MS channels of thermophilic archaea *T. volcanium* and *T. acidophilum* were identified using a functional approach similar to the one used for molecular identification of MscL (Sukharev *et al.*, 1994b). Twenty N-terminal amino acid residues of the MS protein of *T. volcanium* match with 75% identity the start of the open reading frame of a gene encoding MscTA of the related *T. acidophilum* (Kloda and Martinac, 2001c). The channel is nonselective for cations and anions and has a large conductance of ~2.0 nS, comparable to the conductance of MscL and MscMJLR. Similar to all currently known prokaryotic MS channels, MscTA is activated purely by membrane tension in the lipid bilayer (Kloda and Martinac, 2001d). However, membrane tension required for MscTA activation is unusually high compared to other bacterial and archaeal MS channels (Table I) although high negative pressure is also required for the activation of MscL homologues found in *Synechocystis* sp. and *Mycobacterium tuberculosis* (Moe *et al.*, 1998, 2000).

E. Molecular Structure of Prokaryotic MS Channels

The structure of two prokaryotic MS channels, MscL and MscS, has been solved by X-ray crystallography. Rees and coworkers (Chang *et al.*, 1998) solved the 3D oligomeric structure of the MscL homologue from *Mycobacterium tuberculosis* (Tb-MscL). The structure of MscL obtained at 3.5-Å resolution shows a pentameric channel most likely in a closed state. The channel monomer is composed of two α-helical transmembrane (TM) domains, TM1 and TM2, cytoplasmic N- and C-terminal domains, and a central periplasmic domain (Fig. 4A). The transmembrane TM1 helices form a tightly packed bundle funneling to a constriction of ~2 Å at the cytoplasmic side of the channel. The hydrophobic constriction is thought to function as the channel gate. On the basis of functional studies examining permeation of large organic cations through the channel pore (Cruickshank *et al.*, 1997) as well as on spectroscopic studies (Perozo *et al.*, 2002b), the diameter of the MscL funnel at the constriction site was determined to vary between 2 and 30 Å during the channel gating. The overall change in diameter of the channel protein on MscL opening is ~16 Å (Corry *et al.*, 2005), indicating that during opening MscL is undergoing one of the largest conformational changes known in membrane proteins (Fig. 4B). TM1 and TM2 helices are connected by a periplasmic loop that is structurally not well defined. The periplasmic loop is thought to function as a spring resisting the channel

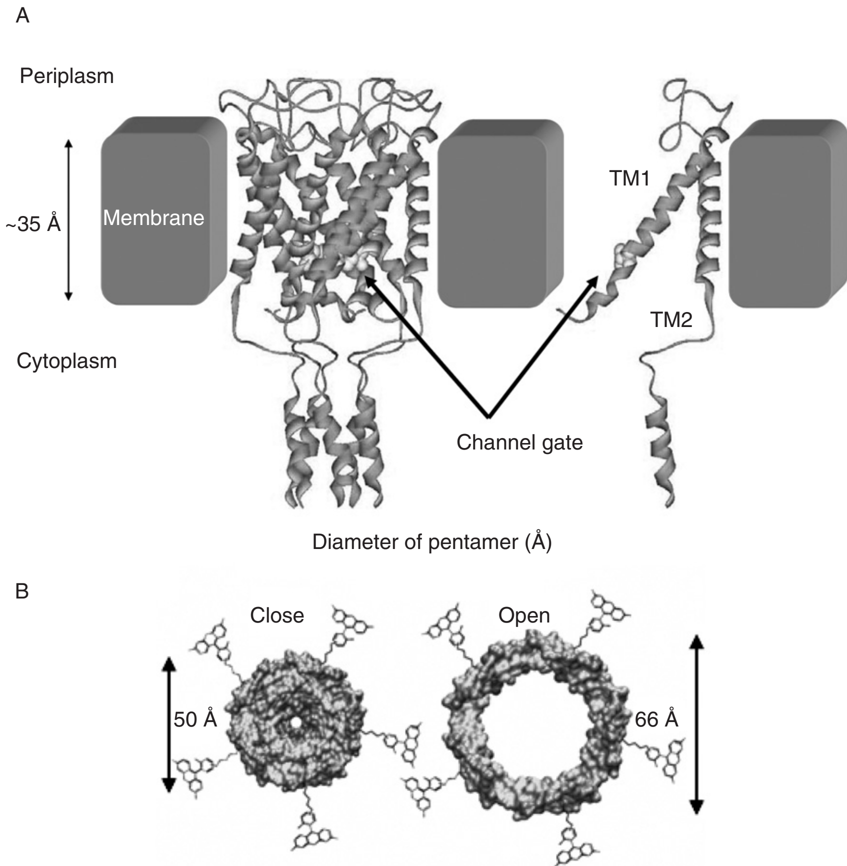

FIGURE 4 (A) The structure of the MscL channel pentamer (left) and a channel monomer (right) from *Mycobacterium tuberculosis* according to the 3D structural model of a most likely closed channel (Chang *et al.*, 1998). The thickness of the membrane bilayer (shown as solid blocks) is ~3.5 Å. The channel gate is formed by a group of amino acids at the cytoplasmic end of the TM1 transmembrane domain. Modified from Oakley *et al.* (1999). Figure based on model 1MSL in Protein Data Bank (http://www.rcsb.org/pdb/). (B) A diagram of a closed and open MscL channel indicating the scale of conformational change involved in channel gating based on a FRET spectroscopic study. Adapted from Corry *et al.* (2005).

opening (Ajouz *et al.*, 2000). A molecular dynamics study of MscL embedded in a curved bilayer suggested that the periplasmic loop could be the first among the MscL domains undergoing structural changes on channel opening (Meyer *et al.*, 2006). The secondary structure of the cytoplasmic N-terminal domain remains unresolved at present. However, amino acid

deletions or substitutions in the N-terminus were shown to severely affect MscL gating (Blount *et al.*, 1996; Häse *et al.*, 1997), suggesting a significant functional role for this structural domain. On the basis of results showing that disulfide coupling is occurring between several highly conserved N-terminal residues that were replaced by cysteines, a model has been proposed in which the N-terminus presents an integral component of the MscL-gating mechanism. In this model, the N-terminus forms a second gate working in accord with the "hydrophobic gate" in the TM1 helix bundle (Sukharev *et al.*, 2001; Betanzos *et al.*, 2002). Nevertheless, the precise role of the N-terminal domain in the MscL gating still awaits to be determined experimentally. According to the 3D crystal structure, C-terminus forms an α-helical bundle (Chang *et al.*, 1998). Its physiological relevance has been put in doubt given the unorthodox crystallographic conditions (pH 3.5) and the abundance of charged groups pointing at the core of the bundle thus indicating a possible instability of its structure. Spectroscopic and molecular dynamics studies, however, demonstrated that under physiological conditions (pH 7.0) the C-terminal cytoplasmic domain also forms an α-helical bundle, located near the fivefold symmetry axis of the channel molecule (Elmore and Dougherty, 2001; Perozo *et al.*, 2001; Martinac, 2004). According to a model based on cysteine-cross-linking experiments, the charged residues of C-terminal helices point toward the aqueous medium and the α-helical bundle is held together by leucine–isoleucine interactions (Anishkin *et al.*, 2003). Interestingly, deletion of the C-terminal bundle was shown not to significantly affect MscL mechanosensation (Blount *et al.*, 1996; Häse *et al.*, 1997; Ajouz *et al.*, 2000), suggesting that this structural domain does not participate in the channel gating. The role of the C-terminus was proposed to be that of a size-exclusion filter at the cytoplasmic side of the MscL pore, preventing loss of essential metabolites (Anishkin *et al.*, 2003). According to this model, the C-terminal domain is stably associated in both closed and open conformations of the channel. A study, however, showed that the stability of the domain is pH dependent (Kloda and Martinac, 2006), indicating that the cytoplasmic α-helical bundle may not only function as a size-exclusion filter but also influence channel gating in a pH-dependent manner.

Rees and coworkers (Bass *et al.*, 2002) solved also the 3D crystal structure of MscS of *E. coli*. Obtained at 3.9-Å resolution, the MscS structure shows that the channel folds as a homoheptamer, which has a large, cytoplasmic region (Fig. 5). Each of the seven MscS subunits contains three TM domains with N-termini facing the periplasm and C-termini at the cytoplasmic end of the channel. According to the crystal structure, the TM3 helices line the channel pore, whereas the TM1 and TM2 helices constitute the sensors for membrane tension and voltage (Bass *et al.*, 2002; Bezanilla and Perozo, 2002).

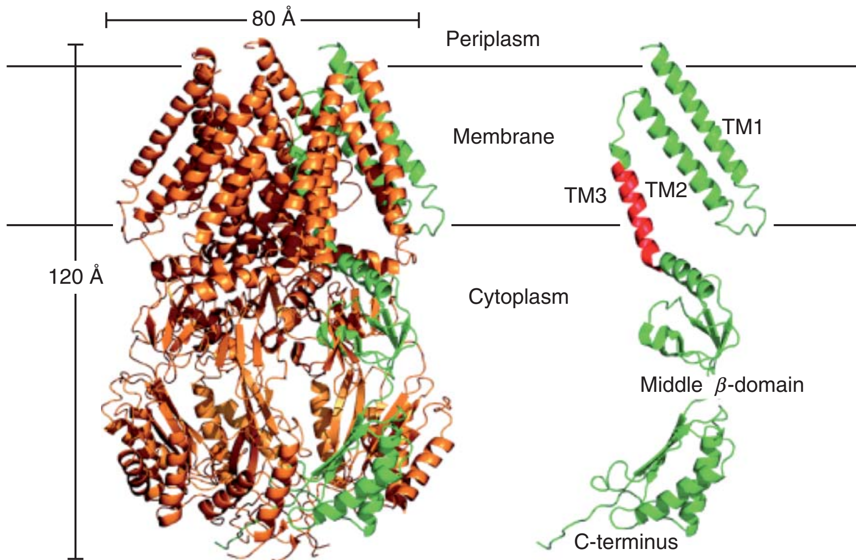

FIGURE 5 The structure of MscS from *E. coli* showing the channel homoheptamer (left) and a monomer (right) based on the crystal structure (Bass *et al.*, 2002) and viewed by PyMol19. Residues 27–280 were resolved. Secondary structural domains and the position of the TM3 transmembrane helix are indicated in the diagram of the monomer. Highlighted in red is a conserved structural motif of glycine and alanine residues in the pore-lining transmembrane helix TM3 essential for gating of prokaryotic MS channels. Reproduced from Martinac (2005a).

Although on initial analysis the 3D structure of MscS was thought to be that of an open channel (Bass *et al.*, 2002), the precise conformation of MscS in the crystal form is controversial at present. A study employing molecular dynamics simulations implied that water and ions cannot pass through the channel pore because of the hydrophobicity of the TM3 residues lining the narrowest portion of the channel pore. This suggested that the crystal structure may reflect an inactive or desensitized functional state rather than the open state (Anishkin and Sukharev, 2004). In another computer simulation study, electric fields were applied to the MscS channel to model the effect of the membrane potential (Spronk *et al.*, 2006). As expected, the application of a potential increased the hydration of the pore and resulted in current flow through the MscS channel. Since the calculated channel conductance was in good agreement with experiment, it was concluded that the MscS crystal structure could be closer to a conducting than a nonconducting state, which would correspond to 11-Å diameter of the TM pore (Bass *et al.*, 2002). According to another molecular dynamics, simulation study the diameter of

the highly hydrophobic MscS channel pore was measured to be 6.5 Å in its narrowest section (Sotomayor and Schulten, 2004). Nevertheless, this study seems to support the notion of the crystallographic structure representing an open state of the channel, because the simulations reported a spontaneous closure of the MscS TM pore when it was permitted to gate spontaneously in a relaxed membrane environment. The channel could be reopened in further simulations by applying membrane tension, which allowed a detailed view of interactions and geometric transformations governing pore closing and opening.

TM1 and TM2 transmembrane domains surround the TM3 helices and are in contact with membrane lipids indicating that they may constitute the sensor for membrane tension. In addition, TM1 and TM2 helices may also underlie modulation of the channel by voltage (Martinac *et al.*, 1987; Sukharev, 2002) because of the presence of three arginine residues in their structure (Bass *et al.*, 2002; Bezanilla and Perozo, 2002). However, the precise contribution of these charged residues to the channel voltage dependence has to be established experimentally.

A large C-terminal cytoplasmic domain is characterized by an interior chamber of 40 Å in diameter, which is in contact with the cytoplasm through multiple openings. Similar to the C-terminal domain of MscL, the cytoplasmic domain of MscS could function as a molecular sieve designed to exclude essential solutes from leaving bacterial cells during a hypoosmotic shock.

F. Bilayer Mechanism and Gating by Mechanical Force

The property of being activated by amphipaths, which are compounds having both hydrophilic and hydrophobic properties and were reported to reversibly change shape of red blood cells (Deuticke, 1968; Sheetz and Singer, 1974), led to a proposal that bacterial MS channels should sense directly membrane tension developed in the lipid bilayer alone (Martinac *et al.*, 1990; Markin and Martinac, 1991). The bilayer mechanism, as this mechanism of the MS channel gating has since been named (Hamill and McBride, 1997), found further support from studies showing that bacterial MS channels preserved their mechanosensitivity after reconstitution into artificial liposomes (Berrier *et al.*, 1989; Delcour *et al.*, 1989; Häse *et al.*, 1995; Blount *et al.*, 1996). This property turned out to be crucial for molecular identification of MscL, the first MS channel identified at the molecular level (Sukharev *et al.*, 1994a, 1997). The bilayer mechanism has since been well documented not only for bacterial channels (Berrier *et al.*, 1989; Delcour *et al.*, 1989; Sukharev *et al.*, 1993, 1994a,b, 1999; Häse *et al.*, 1995), but also for archaeal (Le Dain *et al.*, 1998; Kloda and Martinac,

2001b,c,d) as well as for eukaryotic MS channels (Patel *et al.*, 1998, 2001; Maroto *et al.*, 2005). The property of some eukaryotic MS channels of being gated by bilayer mechanisms was also essential for identification of TRPC-1 as the MscCa in vertebrate cells (Maroto *et al.*, 2005).

Activation of prokaryotic MS channels by pressure (i.e., membrane tension) follows Boltzmann distribution function of the form:

$$P_o = \exp[\alpha(p_{1/2} - p)]^{-1} = \exp\left[\frac{(\Delta G\mathrm{o} - t \cdot \Delta A)}{kT}\right]^{-1} \qquad (1)$$

where P_o is the single channel open probability, α is the slope of ln $[P_o/(1\text{–}P_o)]$ plotted against negative pressure, $p_{1/2}$ is the negative pressure (suction) applied to the patch pipette at which the MS channel is open 50% of the time (i.e., $P_o = 0.5$), ΔG_o is the difference in free energy between the closed and open conformations of the channel in the absence of externally applied membrane tension, ΔA is the difference in membrane area occupied by an open and closed channel at a given membrane tension, and $t{\cdot}\Delta A$ is the work required to keep an MS channel open by external mechanical force at the open probability $0 < P_o < 1$. The conversion from negative pressure (suction) p applied to a patch pipette to membrane tension t is obtained using the Laplace's law $t = p(r/2)$ in which r is the radius of curvature of the membrane patch. This conversion between pressure applied to the patch pipette and bilayer tension in the membrane patch is possible because it was shown that MS channels respond to mechanical forces along the plane of the cell membrane (membrane tension), and not pressure perpendicular to it (Gustin *et al.*, 1988; Sokabe and Sachs, 1990). Membrane tensions required for half activation of MS channels are on the average in the order of several dynes/cm (10^{-3} N/m) (Sachs, 1988).

Since membrane tension t is nearly proportional to the pressure within the range of pressures required for activation of a prokaryotic MS channel reconstituted into a liposome patch and, therefore, is well approximated by the Laplace's law, multiplying $p_{1/2}$ by α (Eq. 1) gives a good estimate of the free energy of MS channel activation ΔG_o (Hamill and Martinac, 2001; Martinac, 2001):

$$\Gamma_{\mathrm{MSC}} = p_{1/2} \cdot \alpha = \frac{\Delta G_o}{kT} \qquad (2)$$

The estimates of ΔG_o obtained for MscL and MscS using Eq. (2) are ~17.0 and ~7 kT, respectively (Martinac, 2001), which is in a good agreement with the patch-clamp results showing that approximately two times less negative pressure is required for activation of MscS compared to MscL in giant spheroplasts of *E. coli* (Berrier *et al.*, 1996; Blount *et al.*, 1996) (Table I).

G. Spectroscopic Studies

Lipid bilayer is at least 10 times more compressible in area than in volume (Hamill and Martinac, 2001). Consequently, any fractional change in area is accompanied by a proportional change in membrane thickness (h) so that

$$\frac{A}{A_0} = -\frac{h}{h_0} \tag{3}$$

where h_0 and A_0 are the unstressed membrane thickness and area, respectively. A 2–4% change in bilayer area with a thickness of 3.5 nm would thin the membrane ~0.1 nm. Given that thinning of a liposome patch would produce a change in matching hydrophobic surfaces of the bilayer and a reconstituted MS channel protein, the assumption was that hydrophobic mismatch could trigger MS channel activation. This is because the energy for transferring a hydrophobic protein surface from an organic solvent to an aqueous environment is ~17 mJ/m^2 (Chothia, 1974). The hydrophobic surface match model derives from the original studies of the gating of gramicidin. This is a small hydrophobic peptide of 15 amino acids that forms cation-selective channels in lipid bilayers by membrane association of one momomer from each monolayer (O'Connell *et al.*, 1990; Harms *et al.*, 2003). Gramicidin exhibits tension sensitivity in lipid bilayers (Elliot *et al.*, 1983) and can switch between stretch activation and stretch inactivation depending on the thickness of the bilayer in which it is reconstituted (Martinac and Hamill, 2002).

Together with the fact that prokaryotic MS channels can be activated by amphipaths known to insert preferentially in one leaflet of the bilayer (Martinac *et al.*, 1990) the assumption that bilayer tension could affect hydrophobic matching between the bilayer and the MS protein led to a spectroscopic and patch-clamp study in which two potential triggers of MS channel gating by the bilayer mechanism were evaluated: (i) protein–lipid bilayer hydrophobic mismatch and (ii) bilayer curvature (Perozo *et al.*, 2002a) (Fig. 6). In this study, structural changes in MscL induced either by hydrophobic mismatch or curving the bilayer by insertion of the amphipath lysophosphatidylcholine (LPC) were examined by combining cysteine-scanning mutagenesis with site-directed spin labeling (SDSL), electronparamagnetic resonance (EPR) spectroscopy, and patch-clamp functional analysis of MscL reconstituted into liposomes. The study demonstrated that hydrophobic surface match could stabilize intermediate conformations of MscL requiring less tension to open the channel in thin bilayers (<18 hydrocarbons per acyl chain) compared to thick bilayers (>18 hydrocarbons per acyl chain), but was insufficient to fully open the channel. However, curving the bilayer by asymmetric insertion of LPC opened MscL without applying membrane

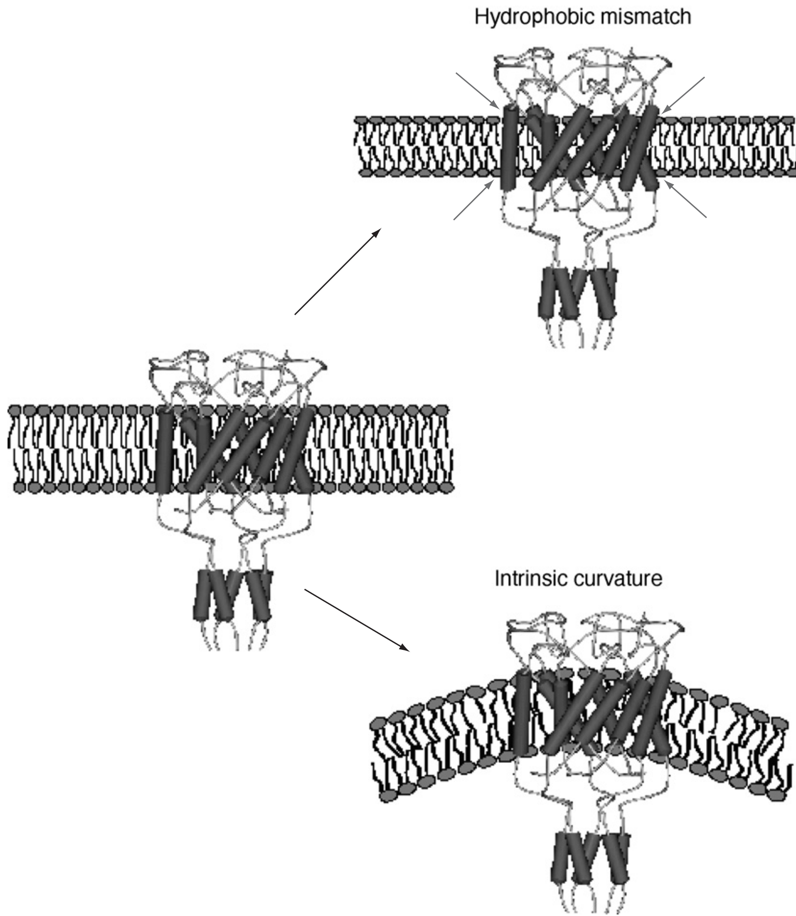

FIGURE 6 Schematic diagram of two possible mechanisms of MscL activation by bilayer deformation forces. Hydrophobic mismatch and bilayer curvature are considered as deformation forces of pressure-induced changes in the lipid bilayer causing conformational changes in MS channels. These changes were studied experimentally by reconstituting purified MscL proteins in liposome bilayers prepared from synthetic phosphatidylcholine lipids of well-defined composition (Perozo *et al.*, 2002a). Reproduced from Martinac (2005b).

tension (Perozo *et al.*, 2002a). Thus, the SDSL EPR spectroscopic study by Perozo *et al.* (2002a) has demonstrated that the mechanism of mechanotransduction in MS channels is defined by both local and global asymmetries in the transbilayer tension profile at the lipid–protein interface, since addition of LPC to one monolayer of liposomes reconstituted with MscL channels created local stresses leading to redistribution of the transbilayer pressure profile in

the lipid bilayer, whereas LPC addition to both monolayers did not. The open state of MscL has a water-filled pore of >25 Å in diameter which is lined by the TM1 helices from the five subunits (Perozo *et al.*, 2002b). This result is consistent with several studies showing that MscL undergoes a large conformational change when opening and closing (Biggin and Sansom, 2001; Gullingsrud *et al.*, 2001; Sukharev *et al.*, 2001; Betanzos *et al.*, 2002; Colombo *et al.*, 2003; Gullingsrud and Schulten, 2003). Conformational changes involved in MscL gating have also very been measured using FRET spectroscopy (Corry *et al.*, 2005). In this study, MscL reconstituted into liposomes was also activated by LPC similar to the SDSL EPR study and the change in FRET efficiency on the channel opening was recorded using a confocal microscope. The diameter of the MscL protein was found to increase by 16 Å on channel activation by LPC (Fig. 4B), which is in excellent agreement with the overall change of the channel diameter estimated by EPR spectroscopy (Perozo *et al.*, 2002b).

These key findings in bilayer-controlled functional properties of MS channels emphasize that the lipid bilayer is much more than a neutral solvent by actively modulating the specificity and fidelity of signaling by membrane proteins (Kung, 2005). A molecular dynamics simulation study by Elmore and Dougherty (2003) reported that MscL protein–lipid interactions were clearly altered by the headgroup changes, leading to conformational differences in the C-terminal region of MscL. The simulations indicated further that hydrophobic matching between MscL and the lipid membrane as well as lipid–protein interactions in general could be more important for proper MscL function and assembly than are protein–protein interactions. This notion has further been supported by another study showing that when hydrophobic residues thought to make contact with the membrane lipid near the periplasmic end of the TM1 or TM2 transmembrane domains of MscL are replaced by hydrophilic residues, MscL apparently loses its mechanosensitivity by becoming unable to open in response to membrane tension. These results suggest that the hydrophobic interaction between the membrane lipid and the periplasmic rim of the MscL funnel is important for the proper function of this channel (Yoshimura *et al.*, 2004).

H. Structural Models of Gating in MscL and MscS

Despite some discrepancies in details of current models of MscL gating, all models include an iris-like rotation and tilt of TM helices as a major structural change during opening of MscL (Betanzos *et al.*, 2002; Perozo *et al.*, 2002b; Anishkin *et al.*, 2005). The TM1 helices, which are packed together to form a right-handed bundle in the MscL pentamer, tilt with

respect to the membrane plane and cause the channel to flatten. Molecular dynamics simulations indicated also that MscL opening should radically reform its tertiary structure (Gullingsrud *et al.*, 2001). Indeed, the flattening of the TM helices leads to opening of a wide channel pore of some 30 Å in diameter (Cruickshank *et al.*, 1997; Sukharev *et al.*, 2001; Perozo *et al.*, 2002b). This basic model of MscL gating is also consistent with the fact that specific hydrophobic mismatch levels stabilize intermediate conformational states of the channel (Perozo *et al.*, 2002a; Elmore and Dougherty, 2003). Instrumental for the changes in helix–helix packing during the close-to-open transition of MscL appears to be the pattern of conserved glycine (Gly) and alanine (Ala) residues near the constriction of the channel pore formed by TM1 helices (Perozo, 2006). In MscS, similar pattern of Gly and Ala residues in the TM3 helix near the constriction point of the channel pore form also a structural motif that is essential for proper channel gating (Fig. 5). The position of the Gly-Ala pattern on the TM3 helix faces is conserved in the MscS family of proteins (Kloda and Martinac, 2002; Pivetti *et al.*, 2003) underlining the significance of this structural motif for gating of prokaryotic MS channels. The gating model of MscS resembles that of MscL. The channel opening is facilitated by slight iris-like rotations and tilt of TM3 pore-lining helices (Edwards *et al.*, 2005). However, the structural changes in MscS are of smaller magnitude compared to that of MscL, which is consistent with approximately three times smaller MscS conductance (Table I). In addition to showing the importance of the Gly-Ala motif, the study by Edwards *et al.* (2005) demonstrated also a remarkable level of plasticity that could be tolerated within MscS primary structure without impairing the channel function (Martinac, 2005a).

For interested readers, further details of structural features and molecular dynamics of MscL and MscS can be found in several reviews (Perozo and Rees, 2003; Blount *et al.*, 2005; Sukharev *et al.*, 2005; Tajkhorshid *et al.*, 2005; Perozo, 2006).

IV. PHARMACOLOGY OF PROKARYOTIC MS CHANNELS

Prokaryotic MS channels can be blocked by submillimolar concentrations of gadolinium (Gd^{3+}) (Martinac, 2001), which is a common blocker of MS channels in many types of cells (Hamill and McBride, 1996). The channels that were probed by Gd^{3+} include MscL and MscS of *E. coli* (Berrier *et al.*, 1992, 1996), MscA1 and MscA2 of *Haloferax volcanii* (Le Dain *et al.*, 1998), MscMJ and MscMJLR of *Methanococcus jannaschii* (Kloda, 2001; Kloda and Martinac, 2002), and MscTA of *T. acidophilum* (Kloda and Martinac, 2001d) (Table I). In contrast to other prokaryotic MS channels, which were blocked by submillimolar concentrations, at least 1 mM of Gd^{3+} was required

to block MscTA. In this context, it is important to mention that Gd^{3+} does not block prokaryotic channels by affecting the channel proteins directly but rather by modifying the mechanical properties of the lipid bilayer surrounding the MS channels (Ermakov *et al.*, 1998). This appears to coincide with comparably larger concentrations of Gd^{3+} required for the MscTA block because a much higher membrane tension corresponding to its unusually high free energy of activation is required for MscTA activation compared to other bacterial and archaeal MS channels (Table I) (Kloda and Martinac, 2002). Besides by Gd^{3+} MscL was also probed by the spider venom peptide GsMtx-4, a novel specific inhibitor of stretch-activated cation-selective MS channels in vertebrate cells (Suchyna *et al.*, 2000; Bode *et al.*, 2001). The peptide neither could block MscL nor did exert any effect on its gating (Liu and Martinac, unpublished).

Both MscS and MscL of *E. coli* are activated by amphipaths, such as chlorpromazine (CPZ), trinitrophenol (TNP), local anesthetics and lysolipids (Martinac *et al.*, 1990; Perozo *et al.*, 2002a), which are known activators of prokaryotic and eukaryotic MS channels (Martinac *et al.*, 1990; Hamill and McBride, 1996; Patel *et al.*, 1998; Kloda and Martinac, 2001a; Qi *et al.*, 2005) (Table I). Similarly, the MS channel of the archaeon *T. volcanii* exhibited an increase in activation by negative pressure in the presence of TNP (Kloda and Martinac, 2001d). MscMJ could also be activated by both CPZ and TNP (Kloda and Martinac, 2001b), whereas MscMJLR was affected by neither of the two (Kloda and Martinac, 2001c). The effect that amphipaths exert on prokaryotic MS channels is indirect, since it is caused by differential insertion of these compounds into the inner and outer leaflet of the lipid bilayer (Markin and Martinac, 1991; Perozo *et al.*, 2002a). In contrast, parabens, which are alkyl esters of *p*-hydroxybenzoic acid and are a class of antimicrobial agents, were shown to open MscL and MscS of *E. coli* by directly interacting with the gate of these channels (Nguyen *et al.*, 2005).

V. FAMILIES OF PROKARYOTIC MS CHANNELS

The finding of MS channels in prokaryotes suggests that these membrane proteins were among the first macromolecules that evolved to facilitate transport of solutes in membranes of protocells. The accessibility of a large number of genome sequences of different bacterial and archaeal evolutionary groups available in various data bases has made possible the analysis of phylogenetic distribution of MS channels from these microorganisms. Multiple sequence alignments of homologues of MscL and MscS revealed that they form separate families of prokaryotic MS channels (Kloda and Martinac, 2002; Pivetti *et al.*, 2003). It has been suggested that MscL-like progenitor molecules might present the prototype of prokaryotic MS genes (Kloda and Martinac, 2002)

and that the two MS channel families may share a common ancestry. An opposite view based on the lack of statistical evidence for a link between the MscL and MscS families argued that the *mscS* and *mscL* genes might have followed separate evolutionary pathways (Okada *et al.*, 2002; Pivetti *et al.*, 2003). Nevertheless, whether an MscL-like progenitor molecule gave rise to a variety of prokaryotic MS channels remains unclear at present because sequence similarity between the highly conserved pore-lining helices in the prokaryotic MS channels, that is, TM1 in MscL and TM3 in the YggB subfamily of MscS proteins, seems to suggest an evolutionary link between MscS and MscL families (Kloda and Martinac, 2002; Pivetti *et al.*, 2003).

A. MscL Family

A group of MscL relatives forms a separate family, which encompasses MS channels of Gram-negative and Gram-positive bacteria, as well as those of a single archaeon, *Methanosarcina acetivorans*, and a fungus, *Neurospora crassa* (Kloda and Martinac, 2002; Kumánovics *et al.*, 2003; Martinac and Kloda, 2003; Pivetti *et al.*, 2003). In terms of their size and sequence, the archaeal and fungal proteins are the most divergent members of the MscL family. In relation to bacterial MscL homologues, they most closely resemble those of Gram-positive bacteria (Pivetti *et al.*, 2003).

B. MscS Family

The MscS channel family is larger than the MscL family. It includes a number of representatives from bacteria, archaea, fission yeast *Schizosaccharomyces pombe* and plant *A. thaliana*, but not from animals (Kloda and Martinac, 2002; Martinac and Kloda, 2003; Pivetti *et al.*, 2003). The MscS relatives are more diverse and vary much more in size and sequence than the MscL relatives. Nevertheless, the MscS family is not ubiquitous, since several organisms with fully sequenced genomes, including Gram-negative chlamydias, Gram-positive clostridia, mycoplasmas, and ureaplasmas, do not encode recognizable MscS homologues (Pivetti *et al.*, 2003).

VI. EARLY ORIGINS OF MECHANOSENSORY TRANSDUCTION

The Earth was formed 4.6 billion years ago and for most of the time since its formation, life on Earth has exclusively consisted of microorganisms (Woese, 1981) (Fig. 7). Given the obvious significance of water for existence of life, the early microbes would have required "emergency valves" for

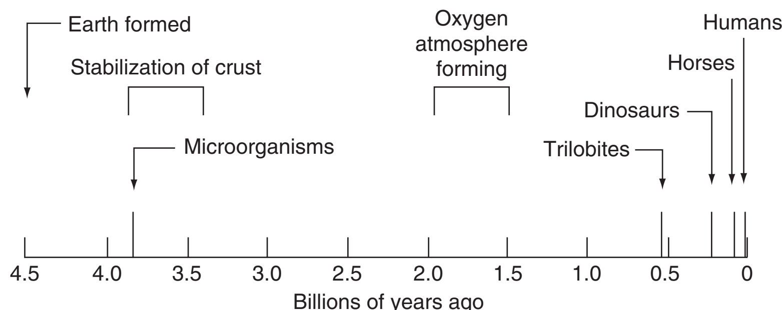

FIGURE 7 Biological time scale for the planet Earth from the time of the Earth's formation 4.6 billion years ago to the time of human origin. The oldest microfossils of prokaryotic cells are ~3.5 billion years of age (Woese, 1981). Reproduced with permission from Woese (1994).

release of osmotic stress to make their survival possible in environments of varying osmolarity. Hence, different authors suggested that the MS channels could have first evolved as cellular osmoregulators (Sachs, 1988; Kung *et al.*, 1990; Morris, 1990; Martinac, 1993; Kung and Saimi, 1995; Sackin, 1995).

A. Physiological Function of MS Channels in Prokaryotic Cells

Bacteria possess multiple adaptation mechanisms enabling them to grow in a wide range of external osmolarities (Wood, 1999; Sleator and Hill, 2001). MS channels, which are located in the cytoplasmic membrane of bacterial cells (Berrier *et al.*, 1989; Levina *et al.*, 1999; Norman *et al.*, 2005) (Fig. 8A), participate in the response to excessive turgor pressure caused by hypotonic conditions. The large conductance and lack of ionic specificity allows the MS channels in prokaryotes to function as "emergency valves" for rapid and nonspecific release of solutes (Fig. 8B). As sensors and regulators of the cellular turgor, they provide a safeguard without which the bacterial cells would lyse. This has unambiguously been demonstrated for MscL and MscS of *E. coli* (Blount *et al.*, 1997; Ou *et al.*, 1997; Levina *et al.*, 1999; Booth *et al.*, 2005). Mutants of *E. coli* lacking both MscL and MscS die on transfer from a medium of high to a medium of low osmolarity (Booth and Louis, 1999; Levina *et al.*, 1999). The third channel, MscM is insufficient alone to protect them. Supporting evidence has been provided for marine bacterium *Vibrio alginolyticus* in which introduction of an *mscL* gene was found to alleviate cell lysis by hypoosmotic shock (Nakamaru *et al.*, 1999). Bacterial cells lacking only MscS or MscL are, however, fully functional. A multiplicity of MS channels may be required to provide a safeguard against the deleterious effects that sudden changes in external

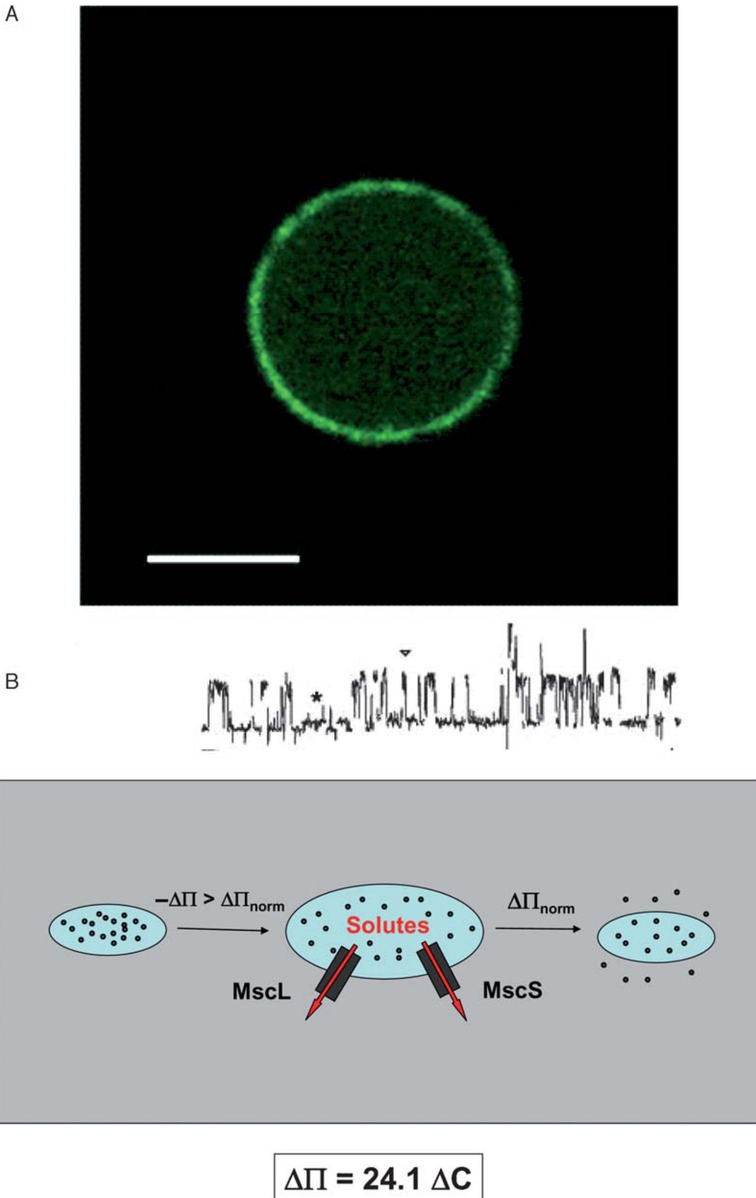

FIGURE 8 (A) Detection of fluorescence and channel activity from MscL channels labeled by green fluorescent protein (GFP) in a giant spheroplast. Confocal image of the giant spheroplast shows that the fluorescence from MscL-GFP is mostly detected in the membrane area, suggesting that MscL-GFP is located in the cytoplasmic membrane. Scale bar = 5 μm.

osmolarity could have on these microorganisms. Hence, the need for channels operating at different levels of cellular turgor appears to be dictated by the different environmental cues of the living habitats in which prokaryotes exist.

Prokaryotic MS channels might also sense changes in turgor pressure during cell division and cell growth given that cell turgor is essential for growth and cell wall synthesis (Csonka and Epstein, 1996). Increase in cell turgor stretches the cellular envelope and causes increase in cell volume, which is required for the synthesis and the assembly of cell wall components resulting in enlargement of the envelope and growth of bacterial cells. Indeed, the expression of MscS and MscL is induced on entry into stationary growth phase when the cells undergo cell wall remodeling and need to relieve the turgor pressure (Stokes *et al.*, 2003).

The physiological role of MS channels in archaea has not clearly been established. However, the archaeal MS channels could be expected to have functions similar to those of their bacterial counterparts. Supporting indirect evidence comes from experiments in which expression of the archaeal MscMJ channel in *E. coli* was shown to impair growth of the bacterium. The growth was partially restored in media of high osmolarity that would cause MscMJ to remain predominantly closed (Kloda and Martinac, 2001b). Although not much is known about turgor pressure in archaea, a partial rescue of *E. coli* cells expressing MscMJ in media of higher osmolarity seems to suggest that cellular turgor could be higher in *E. coli* than in the marine *Methanococcus jannaschii*. Since changes in osmolarity such as ones occurring during flood, drought, or volcanic activity can also be expected to occur in the extreme environments inhabited by archaea, MS channels in these prokaryotic cells may also serve as emergency valves in cellular osmoregulation.

B. Function of MscS-Like Channels in Mechanosensory Transduction in Plants

Plants respond to a number of mechanical stimuli including touch and gravity (Blancaflor and Masson *et al.*, 2003; Braam, 2005) that cause rapid changes in proton and calcium concentration in plant cells. MS ion channels

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Shown below is a patch-clamp recording of MscL-GFP (▽) and MscS (*) channel activity in excised patch of a giant spheroplast. Pipette voltage is +30 mV. Adapted from Norman *et al.* (2005). (B) MS channels in bacteria are essential to maintain cell integrity. Osmotic stress caused by a hypoosmotic shock $-\Delta\Pi$ opens MscL and MscS channels to release excessive turgor pressure. Normally, cell turgor $\Delta\Pi_{norm}$ of a bacterial cell is ~4–6 atm. Depending on the magnitude of the hypoosmotic shock, the turgor pressure may increase well above 10 atm, which without MS channel opening would cause a cell to lyse. $\Delta\Pi$ is osmotic pressure difference in atm (at 22°C), and ΔC is concentration gradient of solutes in moles per liter (osmolarity).

that could mediate these rapid responses have indeed been reported in plants (Falke *et al.*, 1988; Cosgrove and Hedrich, 1991; Ding and Pickard, 1993; Qi *et al.*, 2004). However, none of these MS channels have been identified to date.

Phylogenetic analysis of distribution of prokaryotic MS channels (Section VI) helped to identify MscS-related proteins in the experimental plant *A. thaliana* (Kloda and Martinac, 2002; Pivetti *et al.*, 2003). Out of 10 MscS-like proteins found in this plant two of them, MSL2 and MSL3, have been characterized (Haswell and Meyerowitz, 2006). Both proteins are localized to the inner membrane of the envelope of plastids, which are plant-specific endosymbiotic organelles responsible for photosynthesis, gravity perception, and many metabolic reactions. According to the study by Haswell and Meyerowitz (2006), both MSL2 and MSL3 are involved in control of the plastid size, shape, and possibly division by altering ion fluxes in response to membrane tension occurring during plant morphogenesis. Finding prokaryotic-type MS channels in plants may not be surprising given that plastids in green plants may have originated directly from a cyanobacterium-like prokaryote via primary endosymbiosis (Raven and Allen, 2003; Nozaki, 2005).

VII. CONCLUDING REMARKS

This chapter provides a brief overview of an area of MS channel research that over the last 20 years has gone a log way from its beginnings marked by a discovery of MS channels in bacteria. Recent findings and new developments that are briefly outlined have significantly contributed to our understanding of basic principles and evolutionary origins of mechanosensory transduction in living cells. In the future, we may expect further exciting developments of this research area to continue.

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